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Basic Calibration of UV/ Visible Spectrophotometer

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ABSTRACT

In this work, the calibration of a UV visible spectrophotometer was carried out in order to optimize its performance. A normal calibration method was adopted and Ringbom-Ayre's plot was used to confirm the precision. From the result, it was observed that the curve fitting for the Normal calibration gave the results using calibration model with least square value chosen as performance measure (R^2), the linear model gives the least R - squared (0.997) which is minimal comparing the sum of the residual range thus confirming the accuracy of the results thus confirming the functionality of the instrument.

Keywords: *UV/Vis spectrophotometer, Calibration, Calibration models, performance characteristics, Ringbom-Ayre's plot, Normal calibration, $KMnO_4$*

1. INTRODUCTION

Correct instrument calibration procedure together with a good instrument and a skillful operator gives accurate and precise determination of an unknown sample concentration. For any instrument used for process analysis in the industry, the precision and accuracy of such instrument must be determined so as to give credibility to the response obtained from it especially in a newly installed instrument using a known sample such as Potassium permanganate ($kMnO_4$) which must be of spectra grade standard (100.00$\pm$02).

Instrument Calibration is intended to eliminate or reduce bias in an instrument reading over a range for all continuous value. Random errors affect the precision of an instrument. These errors become minimal when a series of signals is averaged and the relative standard deviation estimated. Standard deviation is a measure of the precision of an instrument. Good precision implies small standard deviation and decreasing relative standard deviation[7].

Ultraviolent-visible spectrophotometer investigates the interaction of light radiation with matter in the ultra violet (200-400) and visible (400-800) range. Potassium permanganate absorbs strongly in the visible range of wavelength between 500 and 550nm on different uv-visible spectrophotometers, it has been reported as having its wavelength of maximum absorption (π_{max}) of normal wavelength as 525nm using spectronic 20, 522nm and Robert Bohman[4] reported his work as 520nm using perkins-elmer.

This work studied the calibration of a newly installed UV-visible spectrophotmeter Jenway 6405 using $KMnO_4$ as a standard with Normal calibration curve and Ringbom Ayre's plot as a confirmation for the level of sprecision. Confirming its readiness and dependability for further work.

Ringbom – Ayre's Plot

Ringbom and Ayres suggested a plot of transmittance against logarithm of the concentration. This gives an 'S' shaped curve with the point of inflexion occurring at 37% transmittance if the system obeys Beer's law. The curve has a region that is relatively linear. The extent of this linear portion indicates directly the optimum range of concentrations and the precision of the determination can be estimated from the slope of the curve[11].

2. MATERIALS AND METHOD

2.1 Safety

The MSDS for detailed information and safety precautions for the standard in addition to the appropriate safety sections in the instrument manual for the necessary requirements was reviewed

2.2 Apparatus

UV/Vis. Spectrophotometer: Jenway 6405 with matched 1cm cuvette, 100ml Standard flask, Test tubes, 10ml pipettes, 1ml pipettes, 50ml beakers, 100ml beakers, Gloves, safety goggle.

2.3 Standard Solutions

A stock solution for standards was made by 0.072g of potassium permanganate ($KMnO_4$) in 250.0cm^3 standard flask. Standard working solutions which contain

respectively 1, 2, 3, 4, 5, 6,7, 8, 9, 10, 20, 40 $mgdm^{-3}$ of potassium permanganate were made by dilution of appropriate aliquots of the stock solution to 100.0cm^3 with deionized water .

2.4 Procedures

In accordance with the operational manual of the spectrophotometer [14], adjustments of the instrument parameter gave optimized conditions for the research work. The absorbance was measured for each solution at 480, 526 and 580nm using the spectrophotometer. The Spectrum of $KMnO_4$ was done to determine the wavelength of maximum absorption at the different concentration by plotting the data for absorbance against wavelength. The data was fitted using "***Grand Optimizer (GO)'*** software. The calibration curve was done by fitting the absorbance against concentration at the wavelength of maximum absorption. This was repeated 5 times.

Ringbom–Ayre's plot was done by fitting transmittance against concentration and transmittance against the logarithm of concentration.

3. RESULT AND DISSCUSION

The absorption spectrum for $KMnO_4$ showed maximum absorption (λ_{max}) at 525nm (fig1). The scanning of the spectrophotometer gave data (Table 1) with performance characteristics: Beer's law validity range of sensitivity 0.039 $AUmg^{-1}dm^3$, with molar absorptivity of $2.468x10^{-7}AUmol^{-1}dm^3cm^{-1}$ and a limit of detection (LOD to 3s.d) $0.002mgdm^{-3}$ Repeatability and level of precision was tested by analyzing 5 replicate samples as was shown by their standard deviation. Standard deviation (SD) (5ppm from the Table 3) = 0.001%Relative standard deviation = 0.0402 < 1%

The curve fitting for the Normal calibration (fig.2) gave a linear model with the least R -squared (R^2) of 0.997 and a correlation coefficient of 0.998.

The result of the study agreed with literature[3,8].

Analysis from the Ringbom- Ayres Plot

A fit of Transmittance Vs Concentration curve follow an Exponential graph (fig.3) and a fit of Transmittance Vs Logarithm of Concentration has a fairly 'S' shaped curve with point of inflexion occurring at 37.5% Transmittance thus obeying Beer's law (fig.4) ,Corresponding to a precision of $-3.469x10^2$ (230/S) relative error [2] [11].

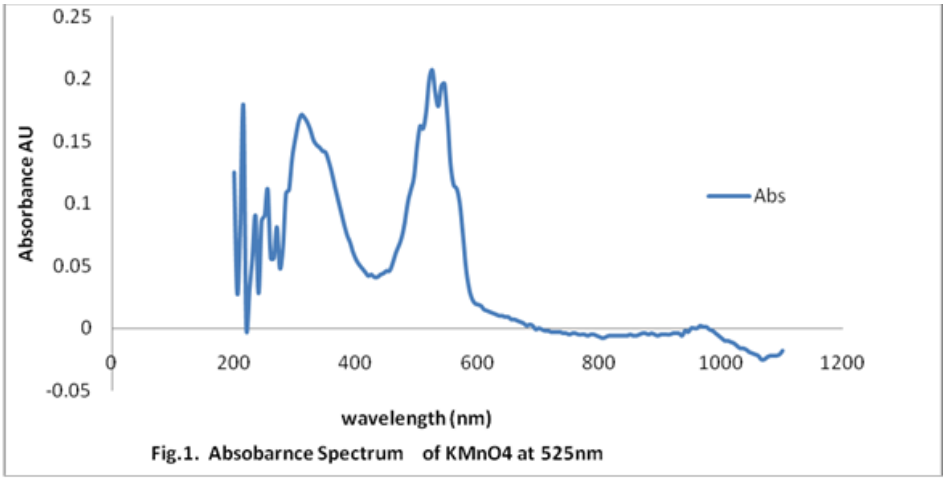

Fig.1. Absobarnce Spectrum of KMnO4 at 525nm

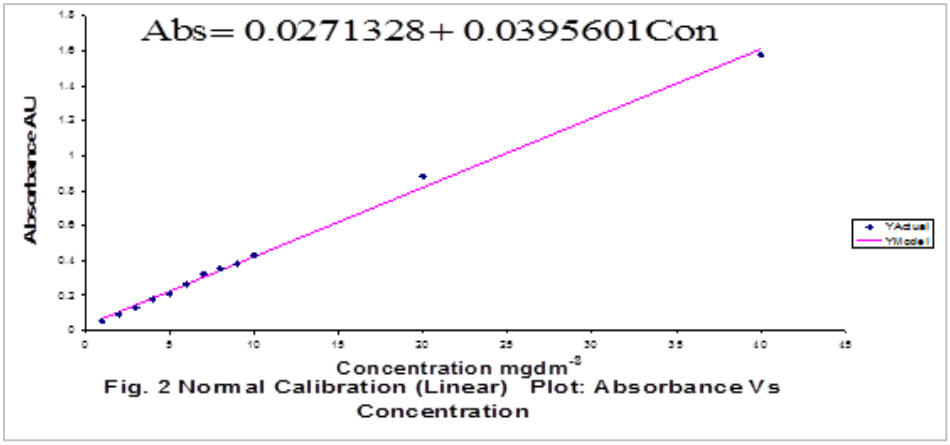

Fig. 2 Normal Calibration (Linear) Plot: Absorbance Vs Concentration

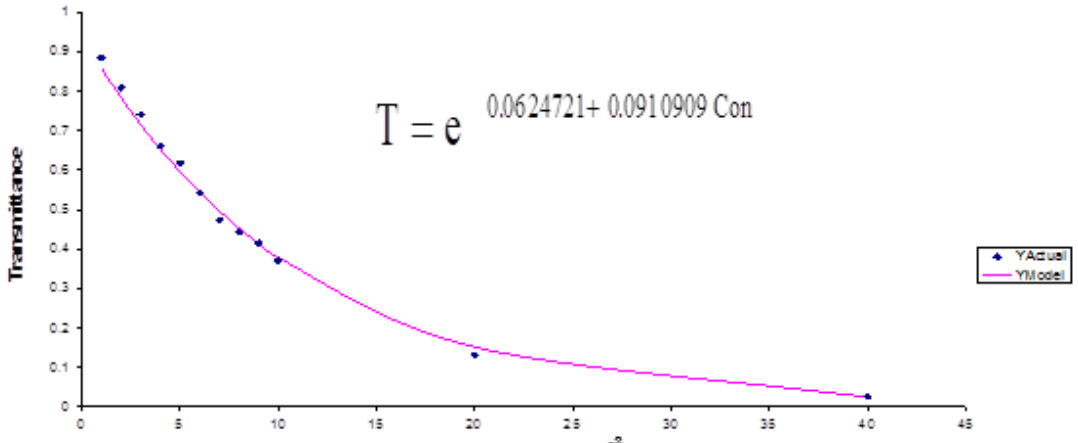

Fig.3 Ringbom- Ayre's plot: Exponential plot : Transmittance Vs Concentration

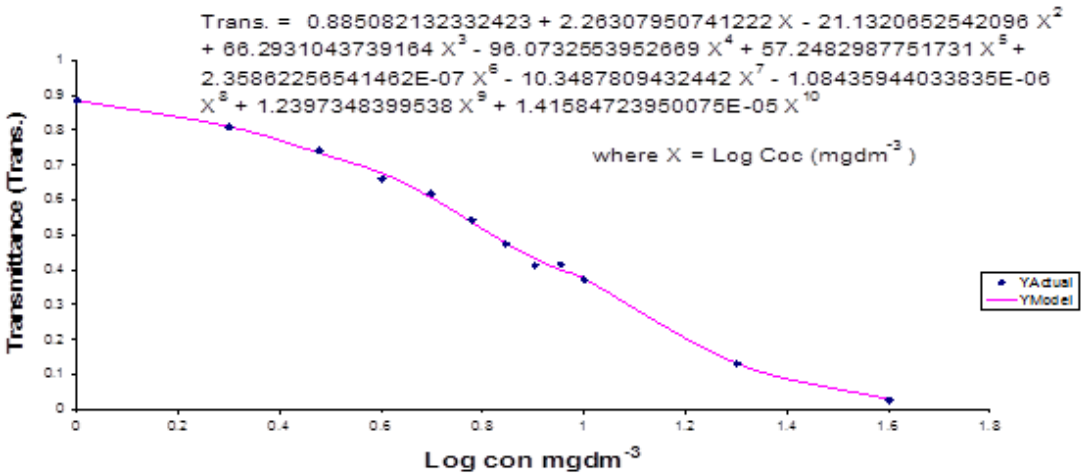

Fig.4 :Ringbom-Ayres's plot: 10th Order Polynomial Regression Plot: Transmittance Vs Logrithim of Concentration

Table 1: Data for the Normal Calibration Curve for KMnO4 at 525nm

CONC ($mgdm^{-3}$)	Absorbance Abs (AU)± S.d	%Transmittance (%T)	Transmittance (T)	Log C
1	0.053± **0**.001	88.51	0.8851	0.0000
2	0.092± 0.000	80.91	0.8091	0.3010
3	0.130± 0.001	74.13	0.7413	0.4771
4	0.180±0.001	66.07	0.6607	0.6021
5	0.209±0.001	61.80	0.6180	0.6990
6	0.265±0.001	54.32	0.5432	0.7782
7	0.324±0.000	47.43	0.4743	0.8451
8	0.354± 0.001	44.26	0.4426	0.9031
9	0.381±0.001	41.59	0.4159	0.9542
10	0.430±0.000	37.15	0.3715	1.0000
20	0.881±0.000	13.15	0.1315	1.3010
40	1.576±0.000	2.66	0.0266	1.6021

***S.d** (5 data points)

Table 2. Regression Analysis Output Summary for Linear Calibration Model

Regression Statistics							
Fitted Model	**No. of Observations**	**Residual Sum of Squares**	**Std. Error**	**R^2**	**Adj. R^2**	**Durbin-Watson**	**Bias of Estimator**
Calibration (Linear)	12	0.0065	0.0809	0.9968	0.9964	2.0698	-5.55112E-16

Analysis of Variance (ANOVA)					
Source	**DF**	**SS**	**MS**	**F Stat**	**p-Value**
Regression	1	2.0078	0.2008	337.7691	0
Residual Error	10	0.0065	0.0006	-	-
Total	11	2.0143	-	-	-

Regression Table				
Variable		**Y**	**X**	**p-Value**
Constant	**Y**	1	0.9983756	0.5506
	X	0.99837564	1	0.0363

Correlation Matrix		
	Y	**X**
Y	1	0.9983756
X	0.99837564	1

Table: 3. Absorbance Readings for potassium permanganate at 525nm

CONC ($mgdm^{-3}$)	**ABSORBANCE (AU)**					**MEAN**	**STANDARD DEVIATION (SD)**	**%RSD = SD/M*100**
	A	B	C	D	E			
0	0.00	0.00	0.00	0.00	0.00	0.000	0.000	0.0000
1	0.053	0.053	0.054	0.054	0.055	0.054	0.001	1.55513
2	0.092	0.092	0.092	0.092	0.092	0.092	0.000	0.00000
3	0.13	0.13	0.129	0.129	0.128	0.129	0.001	0.64757
4	0.181	0.181	0.181	0.179	0.18	0.180	0.001	0.4958
5	0.209	0.208	0.208	0.207	0.207	0.208	0.001	0.40263
6	0.265	0.265	0.264	0.262	0.264	0.264	0.001	0.46392
7	0.324	0.324	0.324	0.324	0.324	0.324	0.000	0.00000
8	0.354	0.352	0.352	0.352	0.354	0.353	0.001	0.31050
9	0.381	0.379	0.381	0.379	0.381	0.380	0.001	0.28812
10	0.43	0.43	0.43	0.43	0.43	0.430	0.000	0.00000
20	0.881	0.88	0.88	0.88	0.882	0.881	0.000	0.10157
40	1.576	1.575	1.576	1.576	1.576	1.576	0.000	0.02838
60	2.062	2.062	2.062	2.060	2.062	2.061	0.000	0.0.0049

4. CONCLUSION

In this work, the calibration of a UV visible spectrophotometer was carried out in order to optimized its performance. A normal calibration method was adopted and Ringbom-Ayre's plot was used to confirm the precision. From the result, it was observed that the curve fitting for the Normal calibration gave the results using calibration model with least square value chosen as performance measure (R^2), the linear model gives the

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REFERENCES

[1] Fundamentals, instrumentation and Technologies of UV/Vis spectroscopy AG/Konrad- Zuse – Strabe 1/07745 Jena / Germay/ info@ analytick-jena.com/

[2] Ayres,G. H. 'Anal. Chem.,21, 652 (1949)

[3] Bohman Robert (2006) "Ultraviolet/Visible (UV – Vis) Spectroscopy of potassium permanganate" November 4th CH EN 4903.

[4] Adeeyinwo C.E and Adesina K. (2002), "Performance 'characterization of Visible Spectroscophotometer in Analysing Silicon." Journal of applied science 5(4): pg 3139 – 3147

[5] Adeeyinwo C.E, (1982) "Flow Injection – Atomic Absorbtion Spectrometry: The use of FIA Dispersions in Normal Calibration Methods in flame AAS"

[6] Engineering Statistics Handbook "Measurement process Characterization"http://www.itl.nist.gov/div898/handbook/mpc/mpc.htm

[7] Barman B. and Baris S. (2009) "Spectrsophotometric determination of Zinc in blood serum of diabetic patients using bis-[2,6-(2-hydroxyl-4-sulpho-1-napthylazo) pyridine disodium salt" Archives of Applied Science Research, 1 (1):pg 74 -83.

[8] Calibration Model Stat Point Technologies, inc 2009 www.statgrahics.com

[9] Geoigian Thomas (2009): "Evaluating goodness – of – fit for linear instrument calibration through the origin international Journal of Environmental Analytical Chemistry Volume 89, Issue 5, pages 383 – 388.

[10] Models 6400/6405 Spectrophotometers operating manual. 64050/REV B /11-99.

[11] Ringbom, A. Z. Anal. Chem. , 115, 332 (1939)